Leica Science Lab Tutorial

Performing a hematoxylin and eosin stain (H&E)

The importance of the hematoxylin and eosin stain in histology and histopathology

The hematoxylin and eosin stain (H&E) is the most widely used stain in histology and histopathology laboratories. When it is properly performed it has the ability to demonstrate a wide range of normal and abnormal cell and tissue components and yet it is a relatively simple stain to carry out on paraffin or frozen sections. In histopathology a high proportion of cases can be diagnosed by an experienced pathologist using an H&E stain alone. Small numbers of slides can be effectively stained manually while in laboratories that have a high throughput it can be performed successfully and consistently by automated slide stainers.

There are a number of different hematoxylin and eosin formulations in popular use each with various advantages and disadvantages. Some laboratories prefer to prepare their own solutions whilst others choose ready-to-use commercial products.

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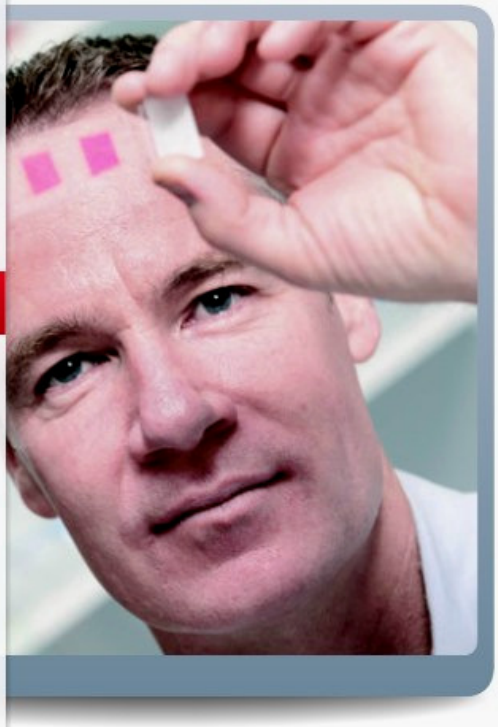
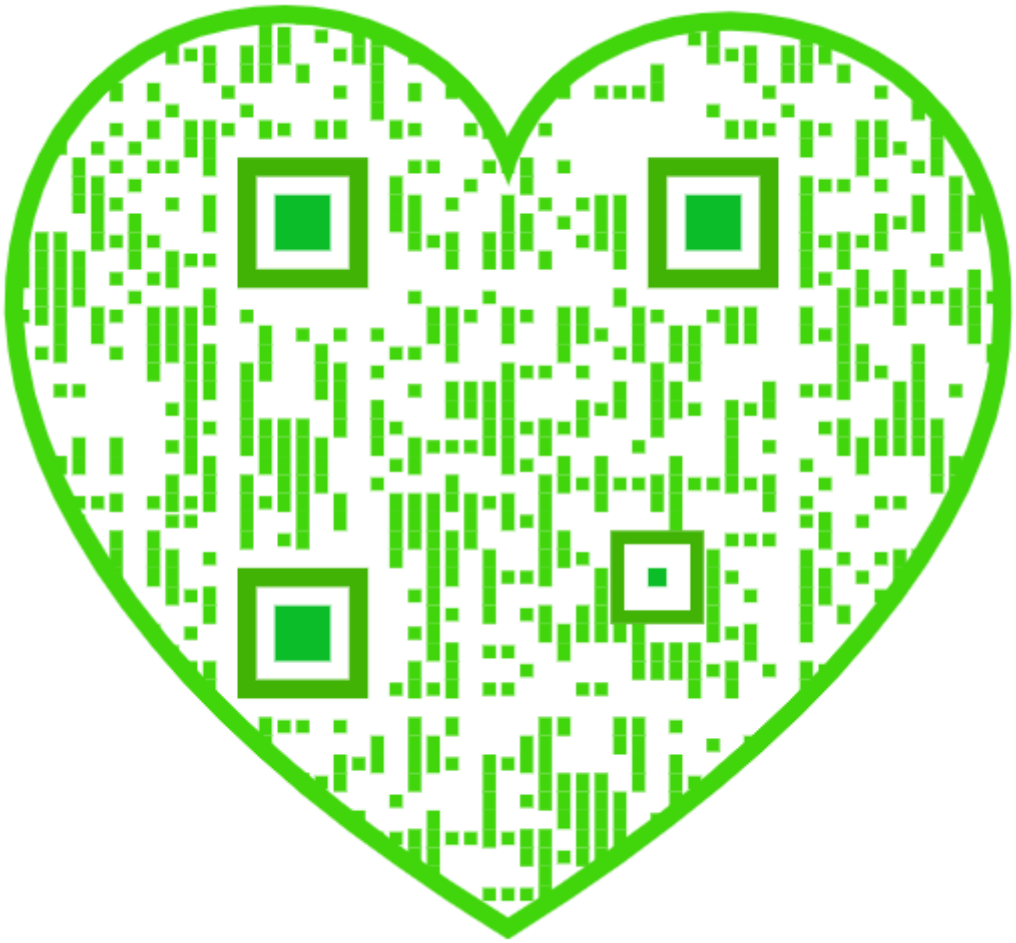




Introduction

The H&E provides a comprehensive picture of the microanatomy of organs and tissues. Hematoxylin precisely stains nuclear components, including heterochromatin and nucleoli while eosin stains cytoplasmic components including cytoplasmic granules, extracellular components including collagen and elastic fibers, muscle fibers and red blood cells. In a high quality H&E there are subtle differences in the shades of color produced by the stains, particularly eosin, and this aids in the detection and interpretation of morphological changes associated with disease.

It is important that people performing and assessing H&E stains for quality are aware of the subtleties of the stain, know what can be achieved when the stain is properly performed with high quality reagents, and know what to look for microscopically. The maintenance of consistent high-quality H&E stains is a fundamental requirement in histopathology laboratories.

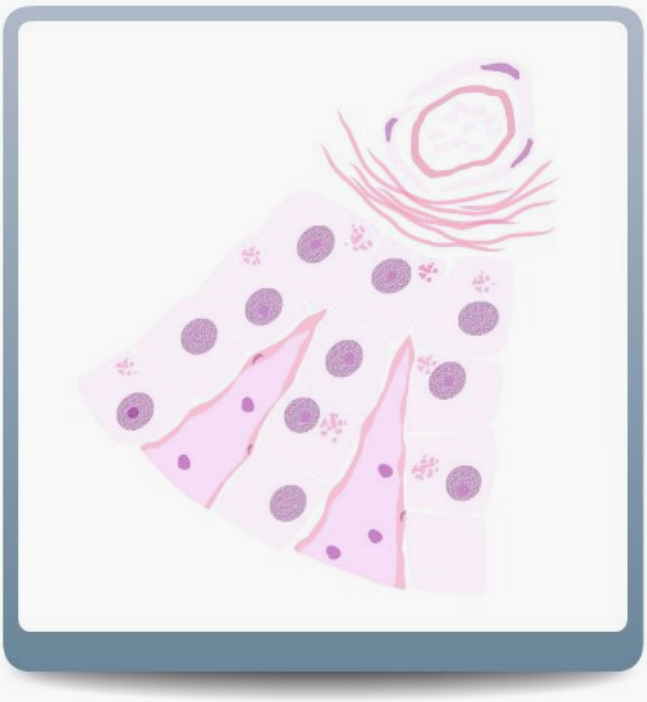
In the following section the basic steps in performing a hematoxylin and eosin stain are outlined and several examples of a completed stain are provided.

Remove the wax

Following the preparation of a paraffin section all the elements are infiltrated with and surrounded by paraffin wax which is hydrophobic and impervious to aqueous reagents. The majority of cell and tissue components have no natural color and are not visible. The first step in performing an H&E is to dissolve all the wax away with xylene (a hydrocarbon solvent).

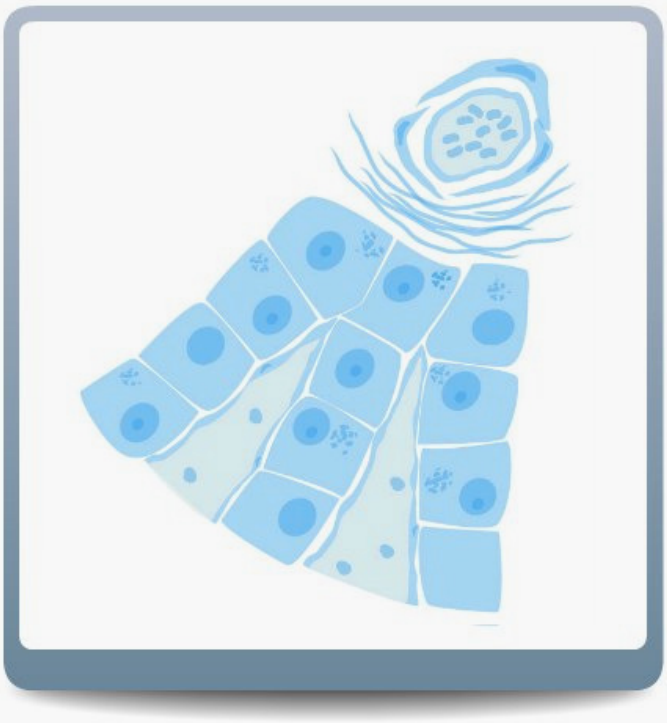
Hydrate the section

After thorough de-waxing the slide is passed through several changes of alcohol to remove the xylene then thoroughly rinsed in water. The section is now hydrated so that aqueous reagents will readily penetrate the cells and tissue elements.



Apply the hematoxylin nuclear stain

The slide is now stained with a nuclear stain such as Harris hematoxylin which consists of a dye (oxidized hematoxylin or hematein) and a mordant or binding agent (an aluminium salt) in solution. Initially this stains the nuclei and some other elements a reddish purple color.

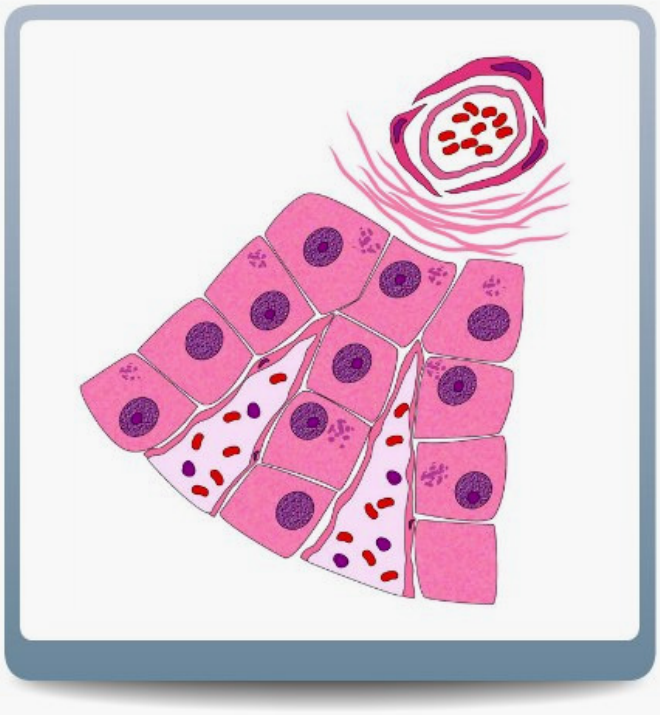


Complete the nuclear stain by "blueing"

After rinsing in tap water the section is "blued" by treatment with a weakly alkaline solution. This step converts the hematoxylin to a dark blue color. The section can now be rinsed and checked to see if the nuclei are properly stained, showing adequate contrast and to assess the level of background stain.

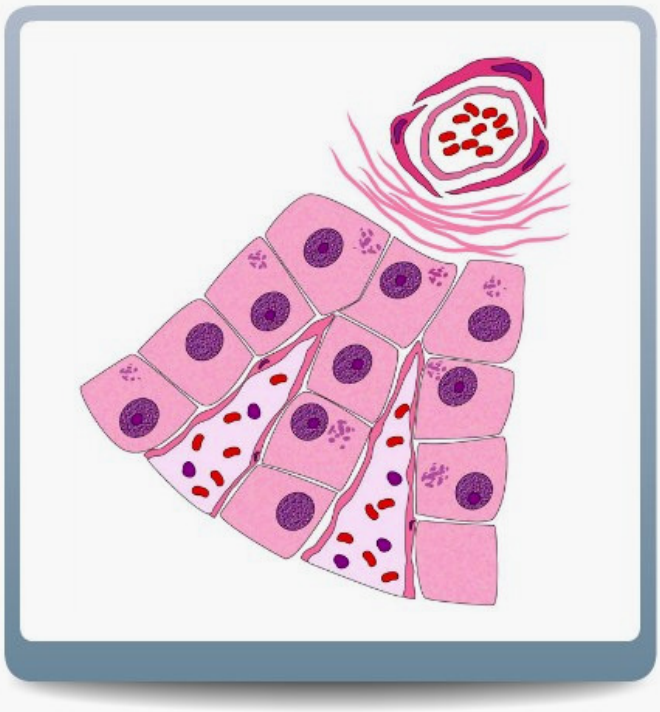
Remove excess background stain (differentiate)

On most occasions when Harris hematoxylin is employed, a differentiation (de-staining) step is required to remove non-specific background staining and to improve contrast. A weak acid alcohol is used. After this treatment blueing and thorough rinsing is again required. Staining methods that include a de-staining or differentiation step are referred to as "regressive" stains.



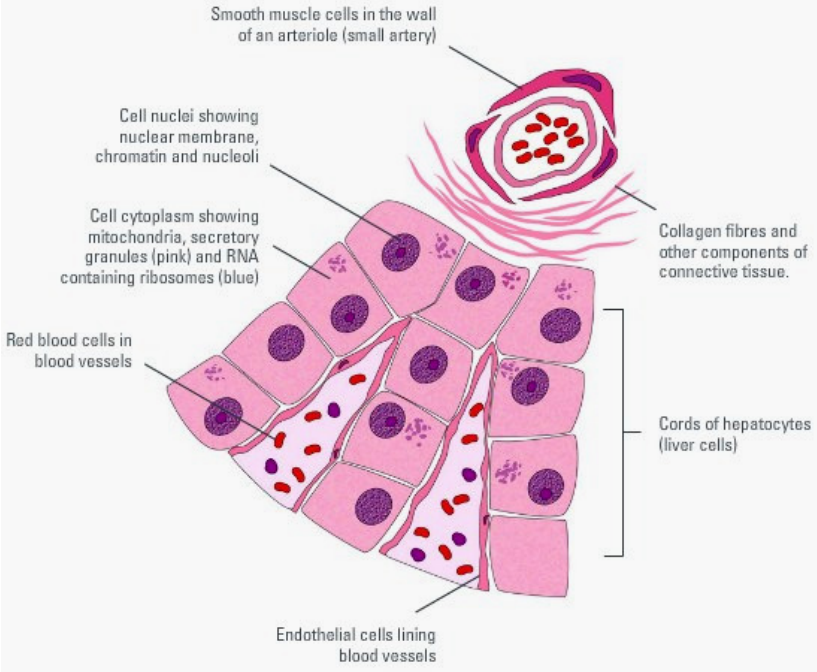
Apply the eosin counterstain

The section is now stained with an aqueous or alcoholic solution of eosin (depending on personal preference). This colors many non-nuclear elements in different shades of pink.



Rinse, dehydrate, clear and mount (apply cover glass)

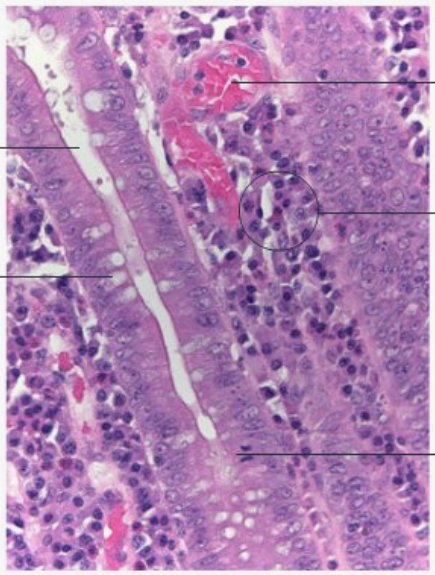
Following the eosin stain the slide is passed through several changes of alcohol to remove all traces of water then rinsed in several baths of xylene which "clears" the tissue and renders it completely transparent. A thin layer of polystyrene mountant is applied followed by a glass cover slip. If the stain and all the subsequent steps have been properly performed the slide will reveal all the important microscopic components and be stable for many years.


Smooth muscle cells in the wall of an arteriole (small artery)
Cell nuclei showing nuclear membrane, chromatin and nucleoli
Cell cytoplasm showing mitochondria, secretory granules (pink) and RNA containing ribosomes (blue)
Red blood cells in blood vessels
Collagen fibres and other components of connective tissue.
Cords of hepatocytes (liver cells)
Endothelial cells lining blood vessels

Examine under the microscope

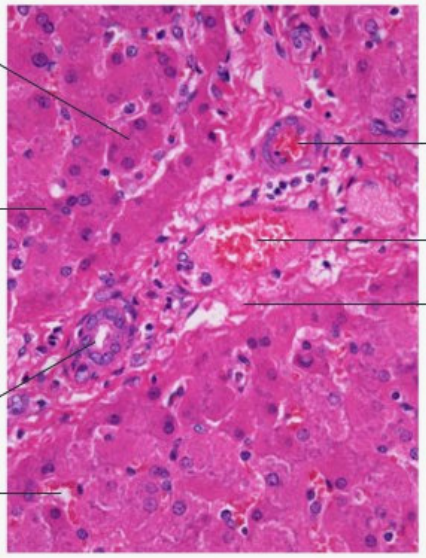
In the small area of liver tissue shown in our demonstration the elements as shown on the left would be stained.

Example of hematoxylin and eosin stains: Small intestine


Blood vessel
Intestinal gland lined with enterocytes
Lamina propria containing various cell types including fibrocytes, lymphocytes, plasma cells and eosinophils (pink granules in the cytoplasm)
Goblet cells (produce mucus)
Mitotic figures (dividing cells)

This micrograph of a paraffin section shows an area from the lining of the small intestine. It has been stained with hematoxylin and eosin (H&E) and demonstrates the features as shown on the left.

Example of hematoxylin and eosin stains: Liver


Cords of liver cells (hepatocytes)
Small artery containing blood cells
Basophilic (blue) aggregations of rough endoplasmic reticulum and ribosomes (containing RNA) in the cytoplasm of hepatocytes
Small vein containing blood cells
Collagen fibres (connective tissue)
Small bile duct lined with epithelium
Sinusoidal blood vessels between the hepatocytes

This is a micrograph of a paraffin section of pig liver. It shows a portion of two hepatic lobules separated by a portal area which contains a small bile duct and several blood vessels which are surrounded by collagenous connective tissue. Visible features are as shown on the left.

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